

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of the claims in the application.

In the claims:

1. (currently amended) A method for stimulating the differentiation of neural crest cells into vascular smooth muscle cells comprising culturing neural crest cells under conditions sufficient for the neural crest cells to differentiate uniformly into smooth muscle cells wherein SM22 α gene expression is induced.
2. (currently amended) The method of claim 1, ~~wherein~~ wherein the neural crest cells are immortalized cells.
3. (original) The method of claim 1, wherein the neural crest cells are cultured in smooth muscle cell differentiation medium.
4. (original) The method of claim 3, wherein said smooth muscle cell differentiation medium comprises the media components listed in Table 1 supplemented with 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μ g/ml), and 25 mM Hepes (pH 7.4).
5. (currently amended) The method of claim 3, wherein said immortalized neural crest cells are ~~first~~ cultured in complete medium prior to culturing in the smooth differentiation medium.
6. (original) The method of claim 5, wherein said complete medium comprises an L-15 CO₂-based medium supplemented with chick embryo extract.
7. (withdrawn) A method for identifying a gene which regulates proliferation or migration of a smooth muscle cells, comprising (i) culturing neural crest cells under culture conditions wherein SM22 α gene expression is induced for a time period sufficient for the neural crest cells to begin differentiation to smooth muscle cells; (ii) identifying genes which are up- or down-regulated under the culture conditions.

8. (withdrawn) The method of claim 7, wherein the step of identifying genes which are up- or down-regulated includes differential display of mRNA from the culture cells with mRNA from non-smooth muscle cells.

9. (withdrawn) The method of claim 7, wherein one or more genes which are up- or down-regulated under the culture conditions are cloned.

10. (currently amended) A method of identifying an agent that modulates differentiation of neural crest cells into ~~which regulates proliferation or migration of~~ smooth muscle cells, comprising: (a) ~~stimulating the differentiation of vascular smooth muscle cells comprising~~ culturing neural crest cells under culture conditions ~~wherein SM22 α gene expression is induced for a time period~~ sufficient for the neural crest cells to begin uniform differentiation to smooth muscle cells; (b) contacting the cells with a test agent; ~~and~~ (c) measuring the ability of the test agent to inhibit the differentiation of the neural crest cells to smooth muscle cells; ~~and (d) detecting cell differentiation phenotypes.~~

11. (currently amended) The method of claim 10, wherein the ability of the agent to inhibit the differentiation of the neural crest cells to smooth muscle cells is measured by detecting the presence or absence of smooth muscle cell markers, wherein the absence of smooth muscle cell markers identifies the test agent as an agent that inhibits the differentiation of neural crest cells into smooth muscle cells.

12. (withdrawn) A method for identifying an agent that modulates proliferation or migration of smooth muscle cells, comprising: (i) identifying a gene product which is up- or down-regulated during differentiation of neural crest cells to smooth muscle cells; (ii) identifying an agent which inhibits or potentiates an activity of the gene product.

13. (withdrawn) The method of claim 12, wherein the gene product is selected from the group consisting of a latent TGF β binding protein, an integrin-linked kinase, an aortic carboxypeptidase, a Torsin, cct ζ , a prothymosin, Limk2, Cca (confluent 3Y1 cell-associated), an interferon activatable protein, an intemexin, a Caspase, AHNAK, Desmoyokin, TSC-36 (TGF

inducible protein), Transcobalamin, a fos-related antigen, an epididymal secretory protein E1 precursor (HE1), a ubiquitin carboxyl-terminal hydrolase, a thyrotropin releasing hormone, and a Decorin.

14. (withdrawn) A method for identifying an agent that inhibits proliferation and/or migration of smooth muscle cells, comprising: (i) identifying a gene product which is up-regulated during differentiation of neural crest cells to smooth muscle cells, the gene product having a biological activity required for proliferation and/or migration of smooth muscle cells; (ii) identifying an agent which inhibits the biological activity of the gene product.

15. (withdrawn) A method for identifying an agent that modulates proliferation and/or migration of smooth muscle cells, comprising: (i) identifying an agent which alters the biological activity of a latent TGF β binding protein (LTBP), and (ii) assessing the ability of the agent to modulate proliferation and/or migration of smooth muscle cells.

16. (withdrawn) The method of claim 15, wherein the agent inhibits interaction of the LTBP with a TGF β complex.

17. (withdrawn) The method of claim 14, wherein the agent inhibits proteolytic cleavage of LTBP.

18. (withdrawn) A method for identifying an agent that modulates proliferation and/or migration of smooth muscle cells, comprising: (i) identifying an agent which inhibits the kinase activity of an integrin linked kinase, and (ii) assessing the ability of the agent to modulate proliferation and/or migration of smooth muscle cells.

19. (withdrawn) The method of claim 15, wherein the agent inhibits phosphorylation of integrin subunits.

20. (withdrawn) A method for identifying an agent that modulates proliferation and/or migration of smooth muscle cells, comprising: (i) identifying an agent which inhibits activation of a LEF-

1/ β -catenin signaling pathway, and (ii) assessing the ability of the agent to modulate proliferation and/or migration of smooth muscle cells.

21. (currently amended) The method ~~of any~~ of claim[[s]] 10, 12, 14, 15, 18 ~~or 20~~, comprising the further step of formulating a pharmaceutical preparation comprising one or more agents identified as able to modulate differentiation of neural crest cells into proliferation and/or migration ~~of smooth muscle cells~~.

22. (withdrawn) A method for modulating proliferation and/or migration of smooth muscle cells comprising contacting the smooth muscle cells with an agent identified by the method of any of claims 10, 12, 14, 15, 18 or 20 as able to modulate proliferation and/or migration of smooth muscle cells.

23. (withdrawn) The method of claim 22, wherein the smooth muscle cells are contacted with the agent in vitro.

24. (withdrawn) The method of claim 22, wherein the agent is administered to animal in order to treat or prevent unwanted proliferation of smooth muscle cells.

25. (withdrawn) The method of claim 24, wherein the agent is administered to animal in order to treat or prevent restenosis.

26. (withdrawn) The method of claim 24, wherein the agent is administered to animal in order to treat or prevent atherosclerosis.

27. (withdrawn) The method of claim 24, wherein the agent is administered to animal in order to maintain an expanded luminal volume following angioplasty or other vessel trauma.

28. (withdrawn) A method for treating or preventing unwanted proliferation of smooth muscle cells in animal, comprising: (i) identifying an agent which inhibits the biological activity of, or inhibits expression of, a gene product which is up-regulated during differentiation of neural crest

cells to smooth muscle cells, the gene product having a biological activity required for proliferation and/or migration of smooth muscle cells; and (ii) administering to animal in need thereof an amount of the agent which is effective to inhibit unwanted proliferation of smooth muscle cells.

29. (withdrawn) A method for treating or preventing abnormal, pathological or inappropriate proliferation of smooth muscle cells in animal, comprising: (i) identifying a gene product which is up-regulated during differentiation of neural crest cells to smooth muscle cells, the gene product having a biological activity required for proliferation and/or migration of smooth muscle cells; (ii) identifying an agent which inhibits the biological activity of the gene product, or which inhibits expression of the gene product, so as to inhibit proliferation of smooth muscle cells; and (iii) administering to animal in need thereof an amount of the agent which is effective to inhibit unwanted proliferation of smooth muscle cells.

30. (withdrawn) A method for identifying an agent that inhibits proliferation of smooth muscle cells, comprising: (i) identifying an agent which inhibits the biological activity of a gene product which is up-regulated during differentiation of neural crest cells to smooth muscle cells, the gene product having a biological activity required for proliferation and/or migration of smooth muscle cells; and (ii) assessing the ability of the agent, or an analog thereof, to modulate proliferation and/or migration of smooth muscle cells.

31. (new) The method of claim 1, wherein the neural crest cells differentiate into a population of cells in which nearly 100% of the cells are smooth muscle cells.

32. (new) The method of claim 1, wherein the conditions comprise culturing in medium comprising 10% fetal bovine serum.

33. (new) The method of claim 1, wherein the conditions comprise culturing in medium that does not include exogenously added active TGF- β 1.

34. (new) The method of claim 1, wherein the neural crest cells are Monc-1 cells.